The Effect of Calretinin Immunostaining as Adjunctive to Hematoxylin and Eosin in Improving Diagnosis of Hirschsprung Disease

A Thesis

Submitted for the partial fulfilment of The Doctorate degree in pediatric surgery

Presented by

Hany Mohammed Embaby M.B.BCH, MSc.

Under supervision of

Prof. Dr. Ayman Ahmed ALBaghdady

Professor of pediatric Surgery, Faculty of Medicine - Ain Shams University

Dr. Hisham Mohammed Abd El Kader

professor of pediatric Surgery department, Faculty of Medicine - Ain Shams University

Dr. Mohammed Sayeed El Sherbeeny

Assistant professor of pediatric surgery, Faculty of Medicine - Ain Shams University

Dr. Marwa Mosaad Shakweer

Assistant professor of pathology, Faculty of Medicine - Ain Shams University

Dr. Mohammed Hisham Soliman

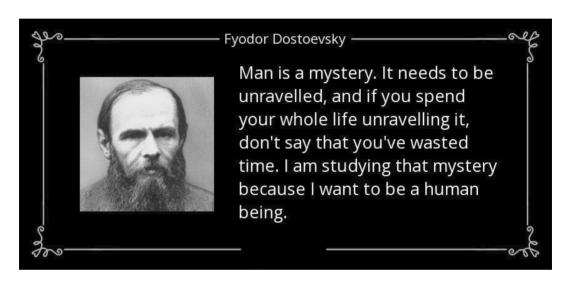
Assistant professor of pediatric surgery, Faculty of Medicine - Ain Shams University

Faculty of Medicine

Ain Shams University

2019





The Crime and the Punishment (Fyodor Dostoevsky, 1880).

Acknowledgment

First of all, all thanks are due to Allah by the grace of whom, this work was possible I would like to express my sincere thanks and deep respect to **Prof. Dr. Ayman** Ahmed Ali Al Baghdady, Professor of pediatric surgery, Faculty of Medicine, Ain Shams University For his help and faithful guidance in the supervision in this thesis and through my life since 2009 no words can express my gratitude toward his fatherhood and continuous guidance and I wish to express my deepest appreciation to Dr. Hisham Mohammed Abd El Kader, professor of pediatric Surgery department, Dr. Mohammed Sayeed El Sherbeeny Assistant professor of pediatric surgery for their faithful support, patience and valuable suggestions and I wish to express my deepest gratitude and profound appreciation to Dr Marwa Shakweer, Assistant Professor of Pathology, Faculty of Medicine, Ain Shams University For her generous remarks, meticulous efforts and devotion. Dr. Mohammed **Hisham Soliman**, Assistant professor of pediatric surgery Faculty of Medicine - Ain Shams University for their faithful support, patience and valuable suggestions. I would like to express my gratitude and thanks to patients and patient caregivers for their cooperation throughout the study.

Acknowledgment

I would like to express my gratitude and thanks to **Dr. Suzan Talaat,** Fellow of Pathology, Ahmed Maher teaching hospital for her continuous encouragement and support during this work and dedication.

I would like to thank the pathology lab technician for their help and dedicated work.

Dedication

I dedicate this work to all my family MY MOTHER, MY FATHER, BROTHER AND SISTERS for their continuous support and guidance. And I should remain indebted to them all my life. I would like to thank my family my father for being with me supporting, my mother without her prayers and asking GOD nothing pass easily and finally the end is the best my little family

Maha Nagib my wife (being with me not an easy job) and my Yassin, Yara and Yasmeen for their unwavering and endless support to see my efforts come to fruition. Thank you for your great support.

List of Contents

Topic	Page
List of Abbreviations	I
List of Figures	II
List of graphs	III
List of Tables	IV
Introduction	1
Aim of work	3
Review of literature	
Embryology of the large intestine	5
Histology of the large intestine	22
Neural Network of the Bowel	33
Physiology of Colon and colonic motility	42
Differential Diagnosis and Workup of Patients with Intestinal Dysmotility	51
Epidemiology of Hirschsprung's disease	57
Clinical picture of Hirschsprung's disease	60
Classification of Hirschsprung's disease	63
Diagnosis of Hirschsprung's disease	65
Calretinin	80
Patients and Methods	84
Results	95
Discussion and conclusions	147
Summary	157

Lists

References	160
Arabic summary	199

List of Abbreviations

	Abbreviation	Full name
1.	HD	Hirschsprung's disease
2.	CR	Calretinin
3.	ENS	Enteric nervous system.
4.	NC	The neural crest
5.	NCCs	Neural crest cells
6.	FG	Foregut
7.	MG	Midgut
8.	HG	Hindgut
9.	SOX10	Sry- box 10
10.	EDNRB	Endothelin receptor b
11.	ENCCs	Enteric neural crest-derived cells
12.	GDNF	Glial cell-line-derived neurotrophic factor
13.	RET	Rearranged during transfection
14.	NTN	Neurturin
15.	ATM	Artemin
16.	PSP	Persephin
17.	GFR	Glycosylphosphatidylinositol-anchored binding component
18.	ECE1	Endothelin-converting enzyme 1
19.	ICC	Interstitial cells of cajal
20.	Kit	Receptor with tyrosine kinase activity
21.	LAPCs	Low-amplitude propagated contractions
22.	HAPCs	High-amplitude propagated contractions
23.	AChE	Acetylcholinesterase
24.	ЕН	Enzyme histochemistry
25.	H&E,	Hematoxylin and eosin
26.	GFAP	Glial fibrillary acidic protein
27.	IHC	Immunohistochemistry
28.	IND	Intestinal neuronal dysplasia;
29.	LDH	Lactate dehydrogenase
30.	NSE	Neuron-specific enolase
31.	PAS	Periodic acid–schiff;
32.	PGP9.5	Protein gene product 9.5
33.	QL	Qualitative
34.	QT	Quantitative
35.	SDH	Succinate dehydrogenase
36.	SMA	Smooth muscle α-actin
37.	TEM	Transmission electron microscopy

Lists

		_
38.	SP	Substance p
39.	VIP	Vasoactive intestinal polypeptide
40.	TCA	Total colonic aganglionosis
41.	IASA	Internal anal sphincter achalasia
42.	SRB	Rectal suction biopsy
43.	GOTHI	General organization of teaching hospitals and institutes
44.	MOHP	Ministry of health and population
45.	PBS	Phosphate buffer saline
46.	DAB	Diamino-benzedine
47.	SPSS	Statistical package for social science

List of Figures

Number	Title	Page
1-	Longitudinal and lateral folding of the embryo	5
2-	Schematic representations of early neural crest development.	8
3-	Trunk anatomy during major stages of neural crest development.	9
4-	Timetable and migratory pathway of NCCs along the embryonic mouse gut. NCC neural crest cell, ENS enteric nervous system.	10
5-	Sources, migratory routes and gene expression in neural crest cells contributing to the ENS.	11
6-	Schematic representation of the embryology of the parasympathetic nerves from the sacral roots S2–S4. This extramural innervation of the distal colon develops during the embryonic weeks (EW) 5–7.	15
7-	Schematic drawing of the embryological colonization of the gut by neuroblasts from the vagus, which arrive at the distal colon during embryonic weeks (EW) 9–12.	15
8-	Layers of the large intestine wall.	22
9-	Diagrammatic description of intestinal layers.	23
10-	Different type of cells in the colonic mucosa.	24
11-	Showing anatomical layers and mucosa.	25
12-	Showing muscularis mucosa and submucosa.	26
13-	Showing Meissner plexus and submucosa.	26
14-	Showing Muscularis propria and ganglion cells.	
15-	Showing Muscularis propria and Muscularis mucosa.	28
16-	Showing subserosa and serosal lining.	29
17-	Showing colorectal transitional zone and squamous zone.	30
18-	Showing anal transition zone and colorectal transition zone.	30
19-	Showing anal transition zone and transition zone between colorectal and squamous zone.	31
20-	Showing perianal skin and appendages.	31
21-	Normal ascending colon with well-developed connective tissue plexus layer and connective tissue nets in longitudinal and circular muscles (picrosirius red staining, 45° cutting angle; $\times 120$).	32
22-	Diagrammatic description to arrangement of neural network.	33
23-	Diagrammatic illustration of sympathetic and parasympathetic ganglia and related ganglia.	34

24-	Diagrammatic illustration of innervation of the colon and related ganglia.	36
25-	Diagrams showing the arrangement of the enteric plexuses.	38
26-	Diagrammatic cross-sections of the wall of the small intestine and the large intestine.	39
27-	Diagram of Stach's plexus (the plexus submucosus extremus) showing the mat of interstitial cells with overlying nerve fibers.	40
28-	Anatomy of intestinal epithelium, ENS, and intestinal muscle layers.	41
29-	Absorptive and storage functions of the large intestine.	43
30-	Schematic representation of the connective tissue net function in muscularis propria.	45
31-	Folding grill formation of connective tissue provides the potential to give way during tensile load.	46
32-	The Neuromechanical Loop hypothesis.	48
33-	Schematic interpretation of the neuro-mechanical loop mechanisms for propulsion of intestinal content initiated and sustained by a bolus.	49
34-	Showing contrast enema for a case of HD demonstrating distal narrow colonic segment and a proximal significantly dilated segment separated by a funnel shaped transition zone.	67
35-	Illustration of the rectum with site of rectal biopsy.	71
36-	Different types of intestinal biopsies in relation to plexus arrangement.	72
37-	The normal rectum showing the physiological, hypoganglionic, or even aganglionic zone above the dentate line that transitions to the normal, more proximal, ganglionic bowel.	72
38-	Layers of the colon and plexus arrangement green arrows Meissner plexus Red arrows Auerbach plexus H&E stain (×40).	73
39-	Meissner plexus H&E staining (×120).	74
40-	Normal ganglion at the Auerbach plexus H&E stain (×400).	75
41-	Showing the muscularis propria and related ganglion cells.	76
42-	Comparison of aganglionic (Left) and ganglionic (Right) rectal biopsy stained by ache.	77
43-	Comparison of aganglionic (left) and ganglion-containing myenteric (right) plexus in an LDH reaction (×300).	78
44-	Immunostaining of rectal suction biopsies for calretinin.	81
45-	Immunostaining of full-thickness biopsies for calretinin.	82
46-	Technique of rectal biopsy. Algorithm to describe method of randomization and examination method.	85 93

48-	Algorithm of study finding in relation to clinical diagnosis.	98
49-	Rectal biopsy of HD case showing absence of ganglion cells in the submucosa and presence of signs of chronic constipation including dilated, congested submucosal blood vessels and hypertrophied muscularis mucosa (H&E X200).	103
50-	The same previous section $400 \times \text{submucosa No ganglia}$ with the presence of thickened nerve trunks.	103
51-	Rectal biopsy of HD case showing absence of ganglion cells in the submucosa and presence of signs of chronic constipation including dilated, congested submucosal blood vessels and hypertrophied muscularis mucosa (H&E X200).	104
52-	The same previous section $400 \times \text{submucosa}$ No ganglia thickened nerve trunks.	104
53-	Rectal biopsy of HD case showing absence of ganglion cells in the intermuscular plane and presence hypertrophied nerve trunks (H&E X200).	105
54-	Rectal biopsy of HD case showing absence of ganglion cells in the intermuscular plane and presence hypertrophied nerve trunks (H&E X400).	105
55-	Shows H&E positive case with ganglia present non-HD case in submucosa $200\times$.	106
56-	Shows H&E positive case with ganglia present non-HD case in submucosa 400× the same above.	106
57-	Shows H&E positive case with ganglia present $$ non-HD case in muscle plane $$ 200× $$	107
58-	Shows H&E positive case with ganglia present $$ non-HD case in muscle plane $$ 400× the same above	107
59-	H&E staining with suspicious result and ganglia presence or absence couldnot judged ×200 sub mucosa.	108
60-	H&E staining with suspicious result and ganglia presence or absence couldnot judged ×400 the same above in the submucosa.	108
61-	H&E staining with suspicious result and ganglia presence or absence couldnot judged $\times 200$ sub mucosa.	109
62-	H&E staining with suspicious result and ganglia presence or absence couldnot judged ×400 the same above in the submucosa.	109
63-	H&E staining with suspicious result and ganglia presence or absence couldnot judged ×200 in the intermuscular plane.	110
64-	H&E staining with suspicious result and ganglia presence or absence couldnot judged ×400 the same above in the intermuscular plane.	110
65-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa 200×.	115
66-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa $400\times$ the same above.	115

67-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa 200×.	116
68-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa $400 \times$ the same above.	116
69-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa $200\times$.	117
70-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa $400\times$ the same above.	117
71-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa 200×.	118
72-	Shows Calretinin positive expression case with ganglia non-HD case in submucosa 400× the same above.	118
73-	Shows Calretinin positive expression case with ganglia non-HD case in intermuscular plane $200\times$.	119
74-	Shows Calretinin positive expression case with ganglia non-HD case in intermuscular plane $400\times$ the same above.	119
75-	Shows Calretinin positive expression case with ganglia non-HD case in intermuscular plane 200×.	120
76-	Shows Calretinin positive expression case with ganglia non-HD case in intermuscular plane 400× the same above.	120
77-	Shows Calretinin negative expression case HD case in intermuscular plane $200\times$.	121
78-	Shows Calretinin negative expression case HD case in intermuscular plane $400\times$ the same above.	121
79-	Shows Calretinin negative expression case HD case in intermuscular plane $200\times$.	122
80-	Shows Calretinin negative expression case HD case in intermuscular plane $400\times$ the same above.	122
81-	Shows Calretinin negative expression case HD case in submucosa plane 200×.	123
82-	Shows Calretinin negative expression case HD case in submucosa plane 400× the same above.	123
83-	Shows suspicious case by H&E in the submucosa.	124
84-	The same case by Calretinin diagnosed in the submucosa ganglia found by its Calretinin expression.	124
85-	Shows suspicious case by H&E in the submucosa.	125
86-	The same case by Calretinin diagnosed in the submucosa ganglia found by its Calretinin expression.	125
87-	Shows suspicious case by H&E in the submucosa.	126
88-	The same case by Calretinin diagnosed in the submucosa ganglia found by its Calretinin expression.	126

Lists

89-	Shows negative case by H&E in the submucosa in non-HD case $\times 200$.	127
90-	The same $\times 400$.	127
91-	Shows positive Calretinin expression but no definitive ganglia morphology non-HD case ×200.	128
92-	The same ×400.	128
93-	Shows the same case suspicious by H&E in the intermuscular plane in non-HD case $\times 200$.	129
94-	The same $\times 400$.	129
95-	Shows the same case suspicious by H&E in the intermuscular plane in non-HD case $\times 200$.	130
96-	The same ×400.	130
97-	Shows suspicious case by H&E in the muscular plane where it shows Calretinin expression with ganglia identified $\times 200$.	131
98-	The same case ×400.	131
99-	Showing suspicious case by H&E in the muscular plane where it shows Calretinin expression with ganglia identified $\times 200$.	132
100-	Below figure (100) the same case ×400.	132
101-	Shows suspicious case by H&E in the muscular plane where it shows Calretinin expression with ganglia identified ×200.	133
102-	The same case as figure $(101) \times 400$.	133